

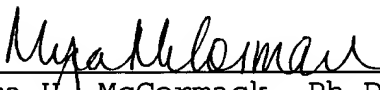
REMARKS

The text of the application as included with this preliminary amendment has been to incorporate Sequence Identification Numbers not present in the original text. No new matter has been added to the specification. A version to show changes made accompanies this amendment.

A sequence listing is provided along with a Computer Readable Form of the Sequence Listing. The Sequence Listing corrects errors in the Sequence Listing consistent with the Notification of Defective Response mailed December 14, 2001. The undersigned hereby states that the Paper Copy and the Computer Readable Form, submitted in accordance with 37 CFR 1.821 et seq. are identical. No new matter has been added by these amendments.

Favorable consideration of this application is respectfully requested.

Respectfully submitted,


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version to show changes made:

The paragraph continuing at the top of page 21 has been amended as follows:

--was obtained with 70 units of AluI on 10 mg of genomic DNA for 20 min. T4 DNA polymerase (Boehringer) and dNTPs (Boehringer) were added to polish the DNA ends. After extraction with phenol-chloroform the digest was size-fractionated on an agarose gel. The genomic DNA fragments with a length of 0.5 to 1.25 kb were eluted from the gel by centrifugal filtration (Zhu et al., 1985). SfiI adaptors (5' GTTGGCCTTTT, SEQ ID NO:115) were attached to the DNA ends (blunt) to facilitate cloning of the fragments into the vector. After ligation of these adaptors to the DNA fragments a second size-fractionation was performed on an agarose gel. The small genomic DNA fragments were cloned upstream of the GAL1 promoter in the vector pGAL1PSiST-1. Qiagen-purified pGAL1PSiST-1 plasmid DNA was digested with SfiI and the largest vector fragment eluted from the gel by centrifugal filtration (Zhu et al., 1985). The ligation mix was electroporated to MC1061 (...) E. coli cells. -

The paragraph beginning at line 10, page 24 has been amended as follows:

--Inverse PCR was performed on 1 µl of the precipitated ligation reaction using library vector specific primers (Figure 1) (3pGALSistPCR: 5' GAG-GGC-GTG-AAT-GTA-AGC-GTG 3' (SEQ ID NO:16) and 5pGALNistPCR: 5'GAG-TTA-TAC-CCT-GCA-GCT-CGA-C 3' (SEQ ID NO:17) for the genomic library; 3pGALNistPCR: 5' TGA-GCA-GCT-CGC-CGT-CGC-GC 3' (SEQ ID NO:18) and 5pGALNistPCR for the cDNA library; all primers from Eurogentec) for 30 cycles each consisting of (a) 1 min at 95 °C, (b) 1 min at 61 (or 57 °C for the cDNA library.

primers), and (c) 3 min at 72 °C. In the reaction mixture 2.5 units of Taq polymerase (Boehringer) with TaqStart antibody (Clontech) (1:1) were used, and the final concentrations were 0.2 µM of each primer, 3 mM MgCl₂ (Perkin Elmer Cetus) and 200 µM dNTPs (Perkin Elmer Cetus). All PCR reactions were performed in a Robocycler (Stratagene).—

The paragraph beginning at line 26, page 24 has been amended as follows:

--PCR analysis is also performed on genomic DNA isolated from the transformants using primers 3pGALSistPCR and 5pGALNistPCR for the genomic library transformants and using primers oligo23': 5' TGC-AGC-TCG-ACC-TCG-AGG 3' ([SEQ ID NO:119](#)) and oligo25: 5' GCG-TGA-ATG-TAA-GCG-TGA-C 3' ([SEQ ID NO:120](#)) (T_{hybr} = 53 °C) for the cDNA library transformants.--

The paragraph continuing at the top of page 28 through line 28 of page 28 has been amended as follows:

--(Perkin Elmer Cetus). Cells were grown to OD₆₀₀ ~ 1.0 and total RNA was prepared using the RNeasy midi kit (Qiagen) according to the manufacturer's instructions. All RNA samples were DNaseI (Boehringer-Mannheim, RNase-free)-treated at 20 U/µg in 50 µl solution for 40 min at ambient temperature, phenol/chloroform-extracted and precipitated. Pellets were dissolved in 20 µl MilliQ water (Millipore) and RNA concentrations were determined spectrophotometrically. First-strand cDNA synthesis was performed in a final volume of 20 µl containing 1x Superscript RT buffer (Life Technologies), 10 mM DTT, 125 µM of each dNTP, 50 µM hexamer primers (Life Technologies) and 1 mg RNA. Mixtures were incubated for 10 min. at ambient temperature and 1 µl was

removed and diluted 1:4 for the non-amplification control (NAC); 20 U Superscript reverse transcriptase (Life Technologies) was added and the reaction was incubated for 1 hour at 42 °C. The enzyme was inactivated for 10 min at 70°C. PCR reactions were set up in triplicate for all genes and contained 5 µl PCR buffer A, 4 mM MgCl₂, 200 µM each of dATP, dGTP, dCTP and 400 µM dUTP, 250 nM fluorogenic probe (for RNR1: 5' TGA-TCT-CAA-AAA-GTG-CTG-GAG-GAA-TCG-GT 3', SEQ ID NO:121), 0.5 U UNG, 1.25 U AmpliTaq Gold, 16.75 µl H₂O, 300 nM of appropriate FORWARD (for RNR1: 5' CGA-CAC-TTT-GAA-ATC-GTG-TGC-T 3', SEQ ID NO:122) and REVERSE (for RNR1: 5' GCA-CCG-GTA-GAA-CGA-ATG-TTG 3', SEQ ID NO:123) PCR primers, 1 µl of the RT reaction mixture. --